Disease Specific Antibodies - Effective Alternative to Antibiotics for Animal Production[1]

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Introduction and Objectives

Enterotoxigenic *Escherichia coli* (ETEC) strains that express K88 fimbriae, cause of morbidity, that negatively affects on feed intake and growth performance in piglets, and considered to be the most important enteric infection in swine industry with major economic significance [1]. As weaning weight of piglet is considered one of the most important factors impacting post-weaning and lifetime growth performance, it should be the goal of swine producers to improve animal health, maximize piglet immunity at weaning and leading to improve their growth performance. Antibiotics have been used traditionally in feed and helped to reduce the detrimental effects of this condition. However, there is mounting pressure to discontinue the use of antibiotics in swine industry, due to the concerns of human health. Therefore, researchers have taken keen interest in searching for an effective alternative to antibiotics that could be used to prevent ETEC infection and to improve animal performance. Passive immunotherapy has been shown to have prophylactic and therapeutic benefits for controlling disease and improving livestock growth performance[2-4]. Avian polyclonal egg antibodies developed against ETEC can be transferred to the recipient by passive vaccination (oral administration) through supplementation of the normal diet of post-weaning piglets, thereby preventing the ETEC disease in piglets and acting as an effective growth promoter[5-7]. Orally administered antibodies provide the advantage of reduced cost and ease of administration for the treatment of enteric infection as well as for improvement of gut health, the key requirement for the improved growth performance, offering effective and sustainable replacements for antibiotics.

The present study was designed to evaluate efficacy of passive vaccination using ETEC K-88 specific Polyclonal Egg antibodies on Prevention of ETEC K-88 infection and improvement of growth performance in piglets. Also, to establish the mechanism of action of ETEC K-88 specific Polyclonal Egg antibodies on ETEC K-88 for adhesion onto porcine intestinal cells *in vitro*.

materials and methods

Production of ETEC K-88 specific Polyclonal Egg Antibodies:

Chickens were hyperimmunized with ETEC K88 fimbrial antigens, following proprietary methods, approved by the Canadian Center for Veterinary Biologics of the Canadian Food Inspection Agency (CFIA). Hyperimmunized eggs were harvested and ETEC K-88 specific polyclonal antibodies were prepared in spay-dried egg powder (SDEP / ABEG), at the CFIA approved facility. The ETEC K-88 specific antibody level in the SDEP/ABEG was determined by ELISA, following methodology approved by the CFIA. The antibody titre against ETEC K-88 antigen was determined to be between 1: 256,000 – 1: 512,000.

Effect of Passive vaccination with Polyclonal ETEC K-88 egg antibodies in nursery diet with or without feed Antibiotics:

- Thirty-six 18 day old piglets (6.3 ± 0.2 kg) were allocated to six treatment groups, blocked by weight and given ad libitum access to feed and water (n=6/group)
- Each group was administered nursery diet supplemented with varying doses of SDEP containing polyclonal antibodies to ETEC K88 and/or antibiotics for 14 days.
- Animals in group 2 received diet without antibiotics, but supplemented with SDEP 1Kg/T, when group 5 received SDEP 4Kg/T, and the group 1 (control) did not receive antibiotics or SDEP. Piglets in group 3 received feed antibiotics + SDEP 1Kg/T, when group 6 received feed antibiotics + SDEP 4Kg/T, while group 4 (control) received only feed antibiotics.
- Animals were weighed at the end of phase 1 (14 d) and phase 2 (28 d), and body weight gains were measured to assess efficacy of passive vaccination with ETEC K-88 antibodies.

Efficacy of Passive vaccination with Polyclonal ETEC K-88 antibodies for Prevention of ETEC K-88 infection in piglets and Improvement of Growth Performance:

- Thirty 18 day old piglets (6.2 ± 0.2 kg) were allocated to five treatment groups, blocked by weight and given ad libitum access to feed and water (n=6/group)
- Group 1 was fed with nursery diet supplemented with 4Kg/T control egg powder obtained from unimmunized chicken, when groups 2-5 were administered varying doses of SDEP/ABEG containing polyclonal antibodies to ETEC K88 for 14 days, day 0-14. Group 2 received ABEG (SDEP) 2 kg/T (0.2%), group 3 received ABEG (SDEP) 4 kg/T (0.4%), when group 4 received ABEG 1 kg/T + Dextrose 1kg/T (Eggstend 0.2%), and the group 5 was fed with ABEG 2 kg/T + Dextrose 2kg/T (Eggstend 0.4%).
- On day 7 post-treatment, all piglets were challenged orally with 5mL of ETEC K-88 at 10^{12} CFU/mL in PBS.
- Animals were monitored to assess effect of ETEC K-88 antibodies for prevention of ETEC K-88 infection by determining incidence as well as severity of diarrhea and shedding of ETEC K-88 bacteria in the rectal swab.
- Animals were weighed on day 7, day 10, day 14 and day 28, post treatment to measure body weight gain due to antibody-mediated improvement of growth performance.

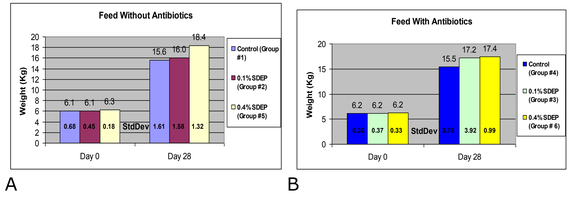
Effect of ETEC K-88 antibodies on adhesion and proliferation of ETEC K-88 bacteria on to porcine intestinal cells:

In order to determine effect of ETEC K-88 antibodies on adhesion and proliferation of ETEC K-88 bacteria, both IPEC-1 and IPEC-J2 porcine intestinal cells in 24-24 well plate were incubated for 60 min at 37°C with 5 x 10^7 ETEC K-88 bacteria in presence of ETEC K-88 antibodies at 0.3 -1.0mg/mL in PBS, when the cells in control wells were incubated with same concentration of control egg powder devoid of K-88 antibodies. After incubation, cells were washed with PBS and the numbers of ETEC K-88 bacteria attached to IPEC-1 and IPEC-J2 cells in presence or absence of ETEC K-88 antibodies were determined under microscope following Giemsa staining to determine adhesion of ETEC K-88. In addition, after washing, the IPEc-1 and IPEC-J2 cells were lysed with 1% Triton X-100, and the viable numbers of ETEC K-88 bound to the cells in each well were determined by plate count to assess colonization of ETEC K-88.

results and discussion

Growth performance of animals fed diet with and without antibiotics and supplemented with SDEP was assessed to determine effect of ETEC K-88 antibodies on growth performance:

- Overall growth performance was improved in all groups supplemented with SDEP, 16.0Kg to 18.4, in groups 2,3,5 & 6, compared with controls 15.5Kg, with antibiotics (Group 4) or 15.6 without antibiotics (Group 1)
- A significant level ($P < 0.05$) of growth improvement was achieved when piglets were fed diet with only SDEP (4 kg/T) without antibiotics (Group 5), compared with a diet that did not contain antibiotics (Group 1), as shown in Figure 1A.
- In both phase 1 (0-14d) and phase 2 (14-28d), 4 kg/T SDEP increased growth performance by 25.3- 27.6% in group 5, over the control group 1. While, 1 kg/T SDEP increased growth performance by 9.2% (in group 2) over the control group 1 in phase 2
- The combination with antibiotics, SDEP improved growth performance in both phase 1 and phase 2. SDEP at 1 kg/T (group 3) increased by 7.5-25%, while SDEP at 4kg/T (group 6) increased by 27.9-16.6%, over the control group 4, as shown in Figure 1B.



A B

Figure 1: Effect of Polyclonal ETEC K-88 antibodies on body weight gain in piglets A. Feed without antibiotics and B. with antibiotics

- Passive vaccination by Oral administration of ETEC K-88 antibodies to piglets following lethal challenge with ETEC K-88 significantly reduced incidence and severity of diarrhea as well as fecal shedding of ETEC bacteria compare to the control, when piglets were fed diet with 2 - 4Kg/T ABEG, as shown in Table 1.
- Overall growth performance was improved in all test groups supplemented with ABEG on day 28 post treatment, compared to the control group, which received 4Kg/T control egg powder devoid of ETEC K-88 antibodies.
- A significant level ($P < 0.01$) of body weight gains were achieved on day 28 post-treatment, when piglets were fed diet with 2 - 4Kg/T ABEG, compared with a diet that did not contain any K-88 antibodies, as shown in Table 2.
- However, piglets fed with only 1Kg/T ABEG in 0.2% AB-DEX did not show any significant reduction of ETEC K-88 infection or any significant improvement in growth performance.

Table -1

Egg Antibodies in Reducing Diarrhea and Fecal Shedding of ETEC in post-weaning piglets

Treatment Groups	Clinical Response		
	Incidence of Diarrhea	Severity of diarrhea (Cumulative Fecal Score)	Average number of E. coli K-88 detected in rectal swabs (10⁵cfu/ swab) at 48-hour post-infection
Control Egg powder (EG)	5/6	42.0	29.66
ABEG - 0.2 %	4/6 N.S.	23.5 p<0.05	1.29 N.S.
ABEG – 0.4%	2/6 p<0.05	19.4 p<0.05	0.68 p<0.05
ABEG-DEX 0.2%	4/6 N.S.	33.2 p<0.05	2.13 N.S.
ABEG - DEX 0.4%	2/6 p<0.05	20 p<0.05	0.16 p<0.05

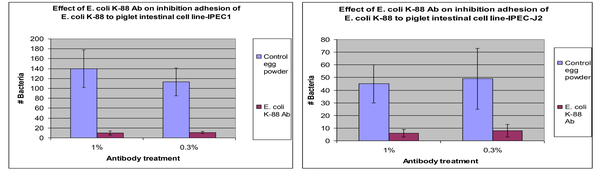
Table -2

Antibodies on improvement of Growth Performance in post-weaning piglets following ETEC K-88 Infection

Study period	Body weight Kg ±SE in different groups				
	EG- (control egg powder 0.4%)	ABEG (0.2% Ab Egg powder SDEP)	ABEG (0.4% Ab Egg powder SDEP)	ABEG-DEX (0.1% Ab + 0.1% dextrose) Eggstend 0.2%	ABEG-DEX (**0.2% Ab +0.2% dextrose**) (Eggstend 0.4%)
Day 0	5.89±0.18	5.96±0.16	5.95±0.15	5.97±0.14	5.96±0.12
Day 7* infection	7.01±0.13	6.84±0.20	7.22±0.18	6.97±0.15	7.11±0.19
Day 10	7.10±0.22	7.12±0.39	7.65±0.25	6.78±0.44	7.69±0.33
Day 14	8.01±0.25	8.61±0.50	9.27±0.60	7.83±0.95	9.41±0.23
Day 28	12.89±0.90	14.59±0.69 **	14.65±1.88 **	13.14±1.03	15.45±1.35 **

** =p<0.01

- Incubation of ETEC K-88 bacteria to ETEC K-88 antibodies at 0.3 to 1% inhibited adhesion of ETECH K-88 bacteria (85-90%) onto porcine intestinal cells, IPEC-1 and IPEC-J2, compared to the incubation with control egg powder that is devoid of specific K-88 antibodies.



A B

Figure 3: Inhibition of adhesion of ETEC K-88 bacteria on to IPEC -1 (A) and IPEC-J2 (B) porcine intestinal cells

conclusions

- It can be concluded that passive vaccination of piglets (fed with nursery diet supplemented) with ETEC K-88 polyclonal antibodies increases body weight of piglets by 2.8 kg over the control in absence of antibiotics. While. ETEC K-88 polyclonal antibodies increases body weight of piglets by 2.0 kg over the control in presence of antibiotics. Antibody dose-dependent effect was only observed when piglets were fed diet without antibiotics.
- Similarly, passive vaccination of piglets (fed with nursery diet supplemented) with ETEC K-88 polyclonal antibodies significantly reduces the infection by ETEC K-88 and improves body weight gain by 2.5 kg over the control, and there is a positive correlation between antibody consumption with protection from ETEC K-88 infection as well as improvement of growth performance.
- Based on the results obtained from *in vitro* studies, it can be concluded that ETEC K-88 antibody mediated effect is due to inhibition of adhesion and colonization of ETEC bacteria onto the intestinal cells.
- Therefore, supplementation of ETEC K-88 polyclonal egg antibodies into swine nursery diet are proved to be as an effective alternative to antibiotic growth promoters for improving animal health, preventing ETEC infection and enhancing animal production.

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